

Synthesis and Antimicrobial Activity of 1-(5-Mercapto-1,3,4-Oxadiazol-2-yl)-2-(Pyridine-2-ylamino)ethanone

(Sintesis dan Aktiviti Antimikrob Sebatian 1-(5-Merkapto-1,3,4-Oksadiazol-2-yl)-2-(Piridine-2-ylamino)etanon

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ABSTRACT

1-(5-Mercapto-1,3,4-oxadiazol-2-yl)-2-(pyridine-2-ylamino)ethanone (3), has been synthesized from 2-(pyridine-2-ylamino)acetohydrazide (2). A yellow colored compound (2) was reacted with carbon disulfide and potassium hydroxide in absolute ethanol to obtain the 1-(5-mercapto-1,3,4-oxadiazol-2-yl)-2-(pyridine-2-ylamino)ethanone. Structures of the synthesized compounds were supported by means of IR, NMR, MS spectroscopic and elemental analysis. The synthesized compounds were evaluated for their antimicrobial activity and were expressed as the corresponding minimum inhibitory concentration (MIC). A brown colored compound (3) with 90% yield has been successfully synthesized. This compound was found to have significant antimicrobial activity with MIC value ranging from 30.2 - 43.2 $\mu\text{g cm}^{-3}$. The findings of the present study indicate that cyclization of hydrazide acid group of 2-(pyridine-2-ylamino)acetohydrazide (2) into 1,3,4-oxadiazole nucleus resulted in increased antimicrobial activity.

Keywords: 1,3,4-oxadiazoles compound; antimicrobial activity; hydrazide acid

ABSTRAK

Sebatian (3), 1-(5-merkapto-1,3,4-oksadiazol-2-yl)-2-(piridine-2-ylamino)etanon telah disintesis daripada sebatian (2), 2-(piridin-2-ilamino)asetohidrazida. Sebatian (2) yang berwarna kuning telah ditindakbalaskan dengan karbon disulfida dan kalium hidroksida dalam etanol kontang untuk menghasilkan sebatian 1-(5-merkapto-1,3,4-oksadiazol-2-yl)-2-(piridine-2-ylamino)etanon. Struktur sebatian yang disintesis disokong oleh cerapan daripada instrumen IR, NMR, spektroskopi jisim dan analisis unsur. Sebatian yang disintesis telah dinilai untuk aktiviti antimikrob dan dinyatakan sebagai nilai penghantaran kepekatan penghalangan minima (MIC). Sebatian (3) yang berwarna perang dengan peratusan hasil 90% telah berjaya disintesis. Sebatian ini didapati menunjukkan aktiviti antimikrob yang signifikan dengan nilai MIC dalam julat 30.2 - 43.2 $\mu\text{g cm}^{-3}$. Kajian ini menunjukkan bahawa proses pensiklikan kumpulan asid hidrazida dalam sebatian 2-(piridin-2-ylamino)asetohidrazida (2) kepada nukleus sebatian 1,3,4-oksadiazol menyebabkan peningkatan dalam aktiviti antimikrobial.

Kata kunci: Aktiviti antimikrob; asid hidrazida; sebatian 1,3,4-oksadiazol

INTRODUCTION

Heterocyclic compounds containing the five-membered nucleus possess a diversity of useful biological effects. The 1,3,4-Oxadiazole (OXD) derivatives are useful targets in the search for antivirals as they have been associated with many types of biological properties such as anti-inflammatory (Amir & Kumar 2007; Narayana et al. 2005; Omar et al. 1996) antibacterial, antifungal activities (Ali & Yar 2007; Zarghi et al. 2005) and inhibit HIV replication (Tan et al. 2006). 2,5-Disubstituted-1,3,4-oxadiazole were disclosed as a broad-spectrum insecticide and acaricide having potential agriculture use (Lanza 1998). In contrast to traditional pesticides, they mainly control the growth and development process of insects by interfering with chitin biosynthesis (Goankar et al. 2006). On the other hand, some of 2,5-disubstituted-1,3,4-oxadiazole derivatives used against 60 tumor cell lines derived from nine cancer cell type. Biological results showed a very interesting anti-

tumor activity against leukemia, colon and breast cancer, for example 3-(5-phenyl-[1,3,4-oxadiazole]-2-yl)-1H-bezoindole (Wagle et al. 2008). However 2,5-disubstituted-1,3,4-oxadiazoles, due to their low solvent solubility, tend to have a non-optimal use rate and formulation difficulties. In an attempt to discover novel leading compounds with high insecticidal and low toxicity, this paper describes the synthesis and antimicrobial activity of new 1-(5-mercapto-1,3,4-oxadiazol-2-yl)-2-(pyridine-2-ylamino)ethanone derivative. The preliminary bioassay tests showed that the synthesized compounds exhibited a significant antimicrobial activity.

MATERIALS AND METHODS

The chemicals used in this work were obtained from Fluka Chemicals Company and they were all pure grade reagents.

SYNTHESIS OF ETHYL 2-(PYRIDIN-2-YLAMINO)ACETATE (1)

Ethyl chloroacetate (0.27 mole, 29 mL) was added drop wise to a stirred solution of 2-aminopyridine (0.27 mole, 26 g), KOH (0.27 mole, 15 g) in absolute ethanol (60 mL). The reaction mixture was refluxed for 5 h and then filtered. The resulting product was dried and recrystallized from ethanol.

SYNTHESIS OF 2-(PYRIDINE-2-YLAMINO) ACETOHYDRAZIDE (2)

A mixture ethyl 2-(pyridin-2-ylamino)acetate (**1**) (0.08 mole, 16 g) and hydrazine hydrate (0.08 mole, 2.48 mL) was refluxed for 3 hours, absolute ethanol (40 mL) was added and the reaction mixture was refluxed for another 3 hours. The separated precipitate was collected and recrystallized from ethanol.

SYNTHESIS OF 1-(5-MERCAPTO-1,3,4-OXADIAZOL-2-YL)-2-(PYRIDINE-2-YLAMINO)ETHANONE (3)

To a solution of 2-(pyridine-2-ylamino)acetohydrazide (**2**) (0.012 mole, 2 g) in absolute ethanol (25 mL), potassium hydroxide (0.012 mole, 0.67 g) and carbon disulfide (0.012 mole, 0.72 mL) were added, respectively. The mixture was refluxed for 17 h or until most of the hydrogen sulfide has been evolved. The solvent was evaporated in vacuum and the separated solid was filtered and recrystallized from chloroform.

ANTIMICROBIAL SCREENING

The antimicrobial activities of the compounds were tested against six pathogenic microorganisms (i) *Staphylococcus aureus* (Gram positive bacteria), (ii) *Streptococcus viridans* (Gram positive bacteria), (iii) *Escherichia coli* (Gram Negative bacteria), (iv) *Fusarium oxysporum* (Fungus), (v) *Alternaria alternata* (Fungus) and (vi) *Alternaria solani* (Fungus). The culture was maintained by the reported procedure (Atlas et al. 1995). The antimicrobial activity of the extracts was qualitatively determined by a modified disc diffusion method. A lawn of microorganisms was prepared by pipetting and evenly spreading inoculum (105-106 c.f.u./cm³) [c.f.u. = colony forming units] onto agar set in petri dishes (the no. of plates were 22), using nutrient agar (NA) for bacteria. Whatman No. 1 filter paper discs of 6 mm diameter were impregnated with the stock solutions of the prepared compounds (100 mg cm⁻³) in DMSO and dried under sterile condition. The dried discs were then placed on the previously inoculated agar surface. The plates were inverted and incubated for 24 h at 30°C. Antimicrobial activity was indicated by the presence of clear inhibition zones around the discs.

INSTRUMENTATION

Elemental C, H, N and S analysis were carried out on a Fison EA 1108 analyzer. The melting points were determined in open capillary tubes using an electrothermal 9300 digital melting point apparatus. The IR spectra of

the compounds were recorded on Shimadzu FTIR-8300 spectrometer as KBr disc. Electron impact MS spectra were obtained on a Shimadzu QP 1000 instrument at 70 eV. The ¹H and ¹³C nuclear magnetic resonance spectra were recorded on Fourier transform Bruker spectrometer, relative to the internal standard tetramethylsilane (TMS) and the chemical shifts are reported in part per million (ppm). ¹H, ¹³C NMR, Mass spectra and elemental analysis were made at Drug and Natural Product Department, University of Vienna, Australia.

RESULTS AND DISCUSSION

The target final product 1-(5-mercapto-1,3,4-oxadiazol-2-yl)-2-(pyridine-2-ylamino)ethanone (**3**) was prepared from reaction of the starting material, 2-aminopyridine, with ethylchloroacetate to yield ethyl 2-(pyridin-2-ylamino)acetate (**1**) which then converted to 2-(pyridine-2-ylamino)acetohydrazide (**2**) through its reaction with hydrazine hydrate. Reaction of this compound with carbon disulfide in the presence of potassium hydroxide gave the final product shown in Figure 1. The purity of the synthesized compounds was checked by TLC using silica gel-G as adsorbent. Table 1 shows the physical and mass analysis data for the synthesized compounds.

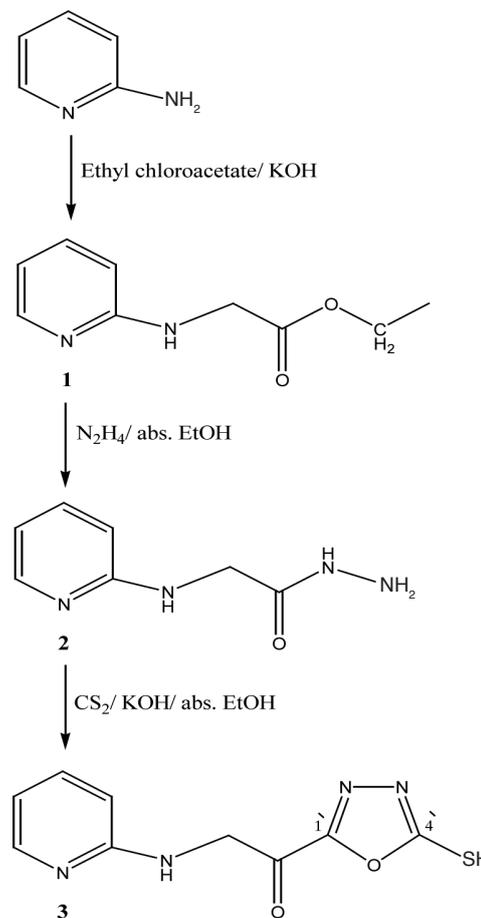


FIGURE 1. Reaction steps for the synthesis of target product

TABLE 1. Physical data for the synthesized compounds

Compound	Color	% Yield	M. P, °C	m/z	Found (Calc.)%			
					C	H	N	S
1	White	97	242-245	194 (100.0%), 195 (11.1%), 196 (1.0%)	61.84 (60.53)	7.27 (6.59)	14.42 (13.68)	-
2	Yellow	85	220-222	166 (100.0%), 167 (7.7%), 167 (1.5%)	50.59 (51.32)	6.07 (6.48)	33.71 (32.91)	-
3	Brown	90	146-149	252 (100.0%), 253 (13.3%), 254 (4.5%), 254 (1.2%)	47.61 (48.13)	4.79 (5.06)	22.21 (22.67)	12.71 (13.42)

INFRA-RED SPECTROSCOPY

The FTIR spectrum of compound (**3**), 1-(5-mercapto-1,3,4-oxadiazol-2-yl)-2-(pyridine-2-ylamino)ethanone, showed the disappearance of bands at 3350-3154 cm^{-1} due to symmetric and asymmetric stretching vibrations of $-\text{NH}_2$ group of compound (**2**). Furthermore, compound (**3**) spectrum showed the appearance of bands at 1620, 1253 and 670 cm^{-1} assigned to stretching absorptions of C=N, C-O-C and C-S groups, respectively (Gaonkar et al. 2006). The IR data of the synthesized compounds are shown in Table 2 and the spectra of compounds (**1**), (**2**) and (**3**) can be shown in Figures 2, 3 and 4.

NUCLEAR MAGNETIC RESONANCE

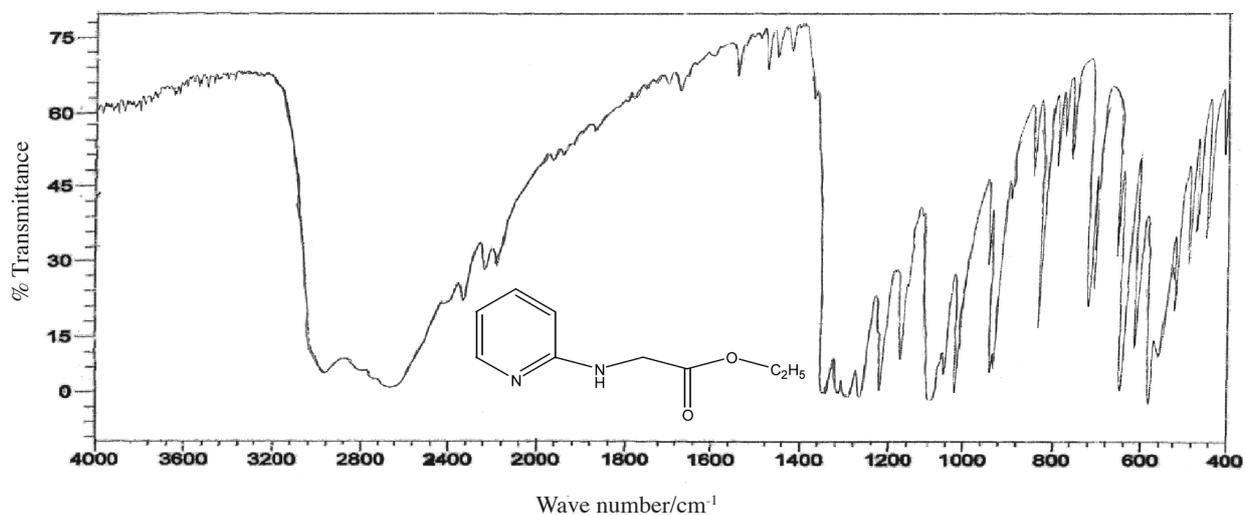
The ^1H NMR spectra for all compounds were recorded in d_6 -DMSO using tetramethylsilane as the internal standard.

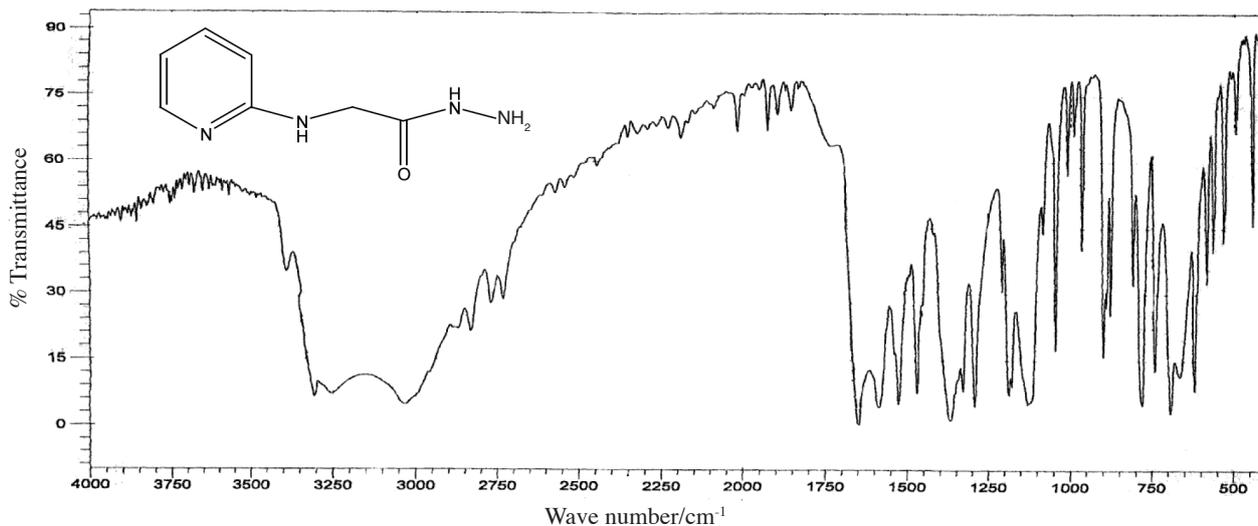
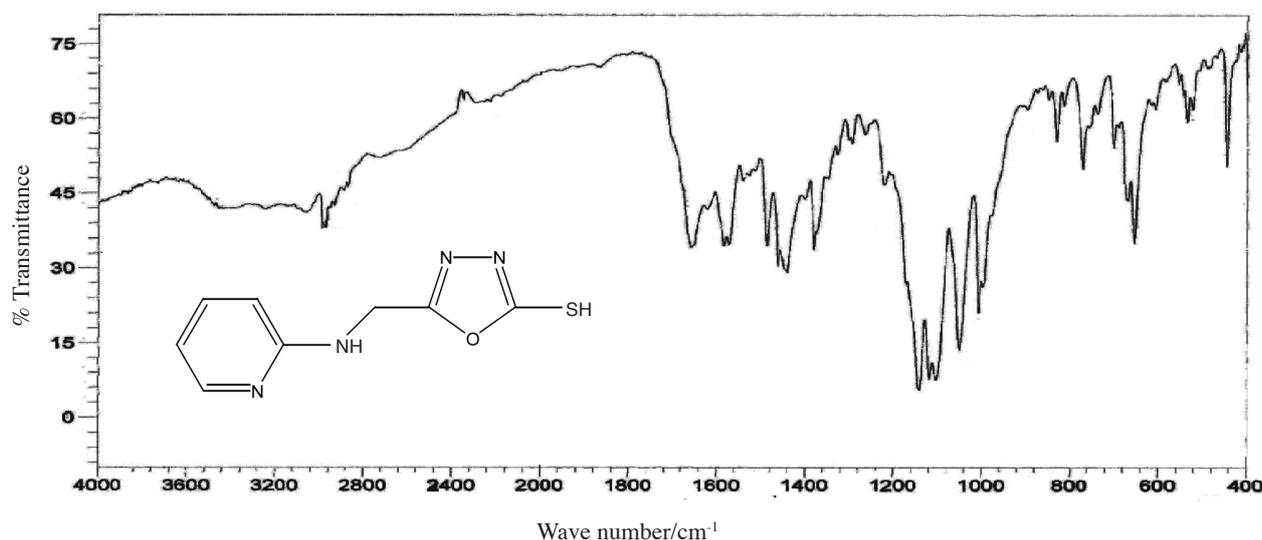
The data are compiled in Table 3. The conclusion drawn from ^1H NMR studies of the synthesized compounds lend to a further support to suggested formation of compound (**3**), 1-(5-mercapto-1,3,4-oxadiazol-2-yl)-2-(pyridine-2-ylamino)ethanone. Compound (**3**) give a single resonance near δ 5.60 ppm attributable to the $-\text{SH}$ proton which further characterized by D_2O exchange. There are a multiple signals of the aromatic protons resonances at δ 6.23-7.60. The spectra also exhibit a singlet peak at δ 8.21 due to N-H group which also further characterized by D_2O exchange (Zarghi et al. 2005).

Table 4 shows the most relevant ^{13}C NMR data. Due to scant solubility of the synthesized compounds, their spectra were recorded in d_6 -DMSO. The $-\text{C}_{(1)}$ - $\text{O}-\text{C}_{(4)}$, aromatic carbons and C=O resonances of compound (**3**) appeared at δ 90.26-92.10, 132.48-137.53 and 160.64 ppm, respectively (Ho & Suen 2009). This observation

TABLE 2. Characteristic absorption bands for the synthesized compounds

Comp.	$-\text{NH}_2$	N-H	C-H aromatic	C-H aliphatic	C=O	C=N	C-O-C	C-S
1	-	3124	3080	2965, 2843	1713	1625	-	-
2	3350-3154	3156	3074	-	1645	1622	-	-
3	-	3130	3065	-	1643	1620	1253	670

FIGURE 2. FTIR spectrum for ethyl 2-(pyridin-2-ylamino)acetate (**1**)

FIGURE 3. FTIR spectrum for 2-(pyridine-2-ylamino)acetohydrazide (**2**)FIGURE 4. FTIR spectrum for 1-(5-mercapto-1,3,4-oxadiazol-2-yl)-2-(pyridine-2-ylamino) ethanone (**3**)TABLE 3. ¹H-NMR spectral data (δ , ppm) for the synthesized compounds

Comp.	-CH ₂ CH ₃	-CH ₂ -	S-H	aromatic protons	N-H	-NHNH ₂
1	2.03-2.06 (q, 2H, CH ₂) 2.65-2.67 (t, 3H, CH ₃)	2.85, s	-	6.95-7.84, m	8.19 (D ₂ O exchangeable)	-
2	-	2.74, s	-	6.64-7.32, m	8.22 (D ₂ O exchangeable)	8.57, 9.12 (D ₂ O exchangeable)
3	-	2.70, s	5.60 (D ₂ O exchangeable)	6.23-7.60, m	8.31 (D ₂ O exchangeable)	-

leads to further support to the suggested structure given to this compound.

ANTIMICROBIAL RESULTS

All the synthesized compounds (**1-3**) were screened for their *in vitro* antibacterial activity against *Staphylococcus*

aureus, *Streptococcus viridans*, *Escherichia coli*, *Fusarium oxysporum*, *Alternaria alternata* and *Alternaria solani* (Table 5). The zone of inhibition was measured in mm and DMSO was used as a blank.

1-(5-mercapto-1,3,4-oxadiazol-2-yl)-2-(pyridine-2-ylamino)ethanone (**3**) has good antimicrobial activity

TABLE 4. ¹³C-NMR spectral data (δ, ppm) for the synthesized compounds

Comp.	-CH ₂ CH ₃	-C ₍₈₎ H ₂ -	-C ₍₁₎ -O-C ₍₄₎ -	aromatic carbons	C=O
1	28.13 (1C, CH ₂) 28.65 (1C, CH ₃)	40.22	-	130.89-137.10	169.45
2	-	41.01	-	133.25-138.14	162.37
3	-	40.58	90.26-92.10	132.48-137.53	160.64

TABLE 5. Quantitative antimicrobial assay results in MIC (μg cm⁻³)

Comp.	<i>Staphylococcus aureus</i>	<i>Streptococcus viridans</i>	<i>Escherichia coli</i>	<i>Fusarium oxysporium</i>	<i>Alternaria alternata</i>	<i>Alternaria solani</i>
1	19.1±0.1	20.1±0.1	21.2±0.2	23.2±0.1	43.1±0.2	7.1±0.1
2	25.2±0.2	38.3±0.1	22.1±0.1	36.2±0.1	18.1±0.2	36.1±0.2
3	41.2±0.1	42.1±0.2	34.1±0.1	43.2±0.1	30.2±0.1	38.3±0.1

with higher value of MIC (Table 5). From structure-activity relationships, introduction of 1,3,4-oxadiazole ring into compound (**3**) significantly increase their biological activity (Husain & Ajmal 2009). Oxadiazole ring system could be incorporated into many more ring systems which itself have their own activity and could lead to more potent and highly active compounds.

CONCLUSIONS

A new 1,3,4-oxadiazole derivative was synthesized by cyclization of acid hydrazide with the objective to develop better antimicrobial agent. The results of biological tests make oxadiazole interesting lead molecules for further synthetic and biological evaluation. It can be concluded that this class of compound certainly hold great promise for discovering safer antimicrobial agents. Many new methods could be developed by using different solvents and substituents.

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